

CLINICAL – MICROBIOLOGY.



Figure 1. Gram stain of *Haemophilus influenzae* from sputum.

HAEMOPHILUS BORDETELLA, AND BRUCELLA.

(Gram-negative-coccobacilli),

Or

(Short - rods) .

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مركز الشامل للخدمات الطلابية

MICROBIOLOGY

❖ **BRUCELLA MELITENSIS.** ←

❖ **BRUCELLA ABORTUS.** ←

❖ **BRUCELLA SUIS.** ←

❖ **FRANCISELLA TULARENSIS.**

❖ **YERSINIA PESTIS.**

❖ **PASTERUELLA MULTOCIDA.**

❖ **ACTINOMYCES ISREALII.**

❖ **NOCARDIA ASTEROIDES.**

❖ **FUNGI CAUSING SYSTEMIC INFECTIONS**
(HISTOPLASMA CAPSULATUM).

GRAM – NEGATIVE RODS RELATED TO ANIMAL SOURCES (ZOO NOTIC ORGANISMS)

Zoonoses are human diseases caused by organisms that are acquired from animals. There are bacterial, viral, fungal, and parasitic zoonoses. Some zoonotic organisms are acquired directly from the animal reservoir, whereas others are transmitted by vectors, such as mosquitoes, fleas, or ticks. There are four medically important gram – negative rods that have significant animal reservoirs : *Brucella* species, *Francisella tularensis*, *Yersinia pestis*, and *Pasteurella multocida* (Table 20-1).

BRUCELLA

Disease *Brucella* species cause brucellosis (UNDULANT FEVER, MALTA FEVER, MEDITERRANEAN FEVER).

Table 20-1 . Gram – Negative Rods Associated With Animal Sources.

Species	Disease	Source of human infection	Mode of transmission from animal to human	Diagnosis
<i>Brucella</i> species	Brucellosis	Pigs, cattle, goats, sheep	Diary products; contact with animal tissues.	Serology
<i>Francisella tularensis</i>	Tularemia	Rabbits, deer, ticks	Contact with animal tissues; ticks	Serology
<i>Yersinia pestis</i>	Plague	Rodents	Flea bite.	Immunofluorescence
<i>Pasteurella multocida</i>	Cellulitis	Cats, dogs	Cat or dog bite	Wound culture

Important Properties *Brucellae* are small gram- negative rods without a capsule. The three major human pathogens and their animal reservoirs are *Brucella melitensis* (goats and sheep), *Brucella abortus* (cattle). And *Brucella suis* (pigs).

Pathogenesis & Epidemiology The organisms enter the body either by ingestion by contaminated milk products or through the skin

by direct contact in an occupational setting such as an abattoir. They localize in the **reticuloendothelial system**, namely, the lymph nodes, liver, spleen, and bone marrow. Many organisms are killed by macrophages, but some survive within these cells, where they are protected from antibody. The host response is granulomatous, with lymphocytes and epithelioid giant cells, which can progress to form focal abscesses and caseation. The mechanism of pathogenesis of these organisms is not well defined, except that endotoxin is involved; ie, when the antigen polysaccharides are lost from the external portion of the endotoxin, the organism loses its virulence. No exotoxins are produced.

Imported cheese made from unpasteurized goats milk produced in either Mexico or the mediterranean region has been a source of *B. melitensis* infection in the United States. The disease occurs worldwide but is rare in the United States because pasteurization of milk kills the organism.

These organisms infect organs rich in Fcγ receptors. Animal-tissues, breast, uterus, placenta and epididymis are rich in Fcγ receptors, but not human-tissues.

Clinical Findings After an incubation period of 1-3 weeks, nonspecific symptoms resembling influenza occur. The undulating (rising - and - falling) fever pattern that gives the disease its name occurs in a minority of patients. Weakness and fatigue commonly occur.

Enlarged lymph nodes, liver, and spleen are frequently found. *B. melitensis* infections tend to be more severe and prolonged whereas those caused by *B. abortus* are more self-limited. Osteomyelitis is the most frequent complication. **SECONDARY SPREAD FROM PERSON TO PERSON RARE.**

Laboratory Diagnosis Recovery of the organism requires the use of enriched culture media and incubation in 10% CO₂. **THE ORGANISMS CAN BE PRESUMPTIVELY IDENTIFIED BY USING A SLIDE AGGLUTINATION TEST WITH BRUCELLA ANTISERUM**, and the species can be identified by biochemical tests. If organisms are not isolated, analysis of a serum from the patient for a rise in antibody titer of at least 1:160 in the convalescent - phase serum sample is diagnostic.

Treatment The treatment of choice is **TETRACYCLINE PLUS GENTAMICIN**. There is no significant resistance to these drugs.

Prevention Prevention of Brucellosis involves pasteurization of milk, immunization of animals, and slaughtering of infected animals. There is no human vaccine.

- * → 1/ Rapid-Plate-Agglutination-Reaction. 2/ Rose-Bengal card-test 3/ Wright's Tube-Agglutination-Reaction.



Phase-Medium → 30 days at +37°C incubation.

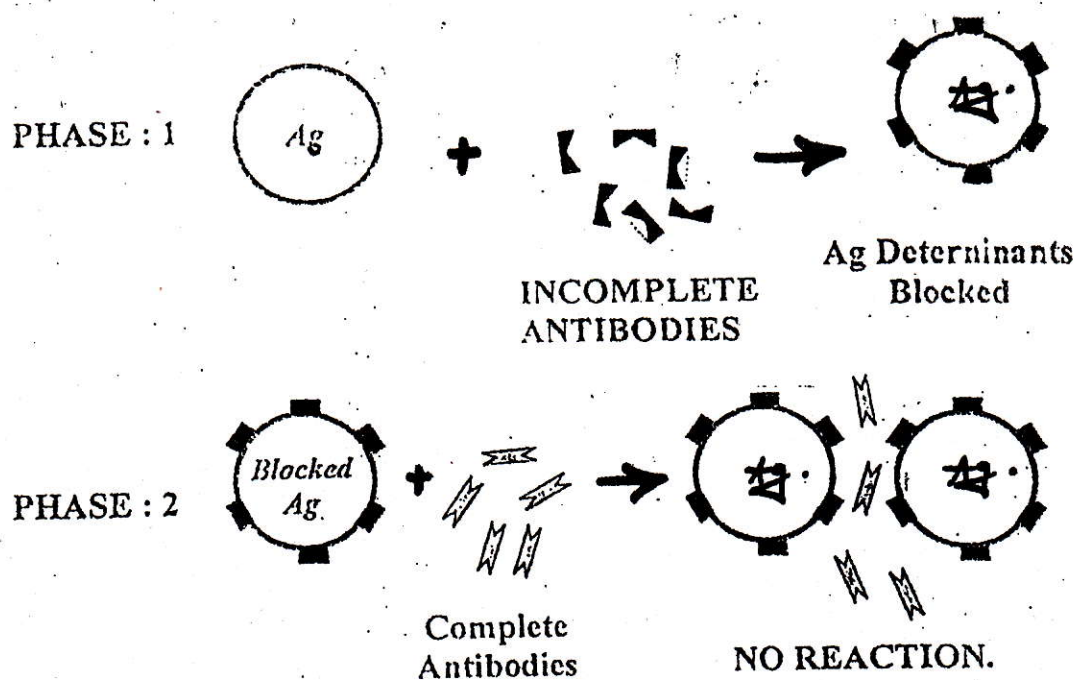
in animals they lead to abortion, sterility and long-time infertile

Prozone reactive :

A prozone reactive can occur when the anti - cardiolipin antibody titre is very high, e.g. in secondary syphilis . Excess antibody prevents normal antibody antigen reactivity, which may result in a test being falsely reported as non - reactive. With experience most prozone reactions can be recognized by their roughness or abnormal grainy appearance, however initial testing of sera undiluted 1 in 20 will pickup any prozone reaction . (Fig 21) .12 q

Fig . 21

PROZONE - REACTION :



PROZONE PHENOMENON: -

Occurs in the serodiagnosis of brucellosis and syphilis where the patient's incomplete antibodies (IgA), non-agglutinating and non-precipitating-antibodies have speed affinity to block the antigens determinants therefore precipitation and agglutination between complete antibodies and the antigen do not occur at low-dilutions of the serum, but occur at high-dilutions.

Prozone-phenomenon carries a great importance in the diagnosis of brucellosis as well as the diagnosis and epidemiology of syphilis. 12.

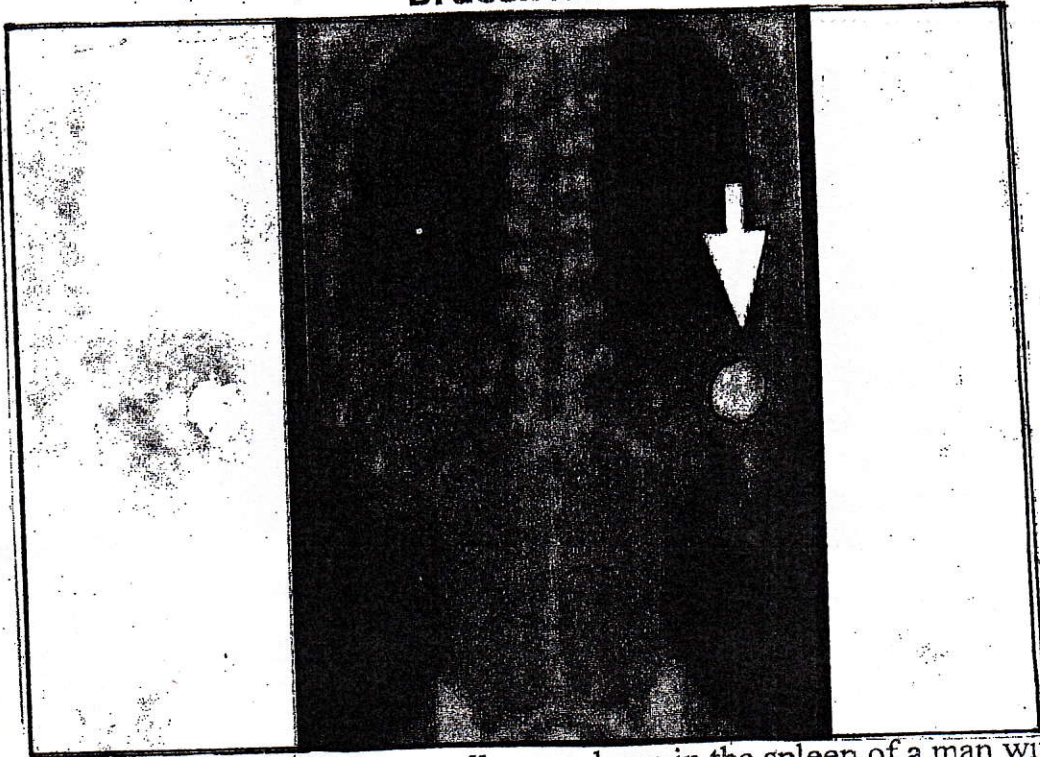
Incorrect performance of test:

The manufacturer's instructions for performing qualitative and quantitative tests must be followed exactly, including the use of controls. Specimens and reagents must be used at the correct testing temperatures. Reactivity is decreased at temperatures below 23 °C and increased at temperatures over 29 °C. Other sources of error include the use of contaminated reagents, testing samples that are haemolyzed, lipaemic or contaminated, adding an incorrect volume of antigen, or rotating tests at an incorrect speed or for an incorrect length of time. 12.

DIFFERENCES BETWEEN TYPHOID - FEVER AND BRUCELLOSIS :-

Differences	TYPHOID - FEVER.	BRUCELLOSIS.
1/ Disease:	ENTERIC - FEVER	BRUCELLOSIS.
2/ Source of infection:	MAN. (Pure-human infection).	ANIMALS → ZOONOSIS.
3/ Causative organism:	Salmonella typhi	a/ Brucella - abortus. b/ Brucella - melitensis c/ Brucella - Anir.
4/ FEVER:	STEPLADDER-PYREXIA.	UNDULANT - FEVER.
5/ Bacteremia:	IN the first-week.	Prolonged - Bacteremia.
6/ Blood - cultures:	Positives during First-week	Difficult to isolate.
7/ Stool cultures:	Positives from the 2 nd week onwards.	Negatives.
8/ Serology:	Gives FALSE-POSITIVE reactions.	The only reliable-test.
9/ SKIN-tests:	NON.	Brucellin - SKIN-Test is of LITTLE - value.
10/ Complications:	a/ INTESTINAL - Perforation, Peritonitis and death. b/ Typhoid - Meningitis	a/ Osteomyelitis is the most frequent
11/ Treatment:	a/ Chloramphenicol OR b/ CIPLOX.	Tetracycline + Gentamycin.
12/ Vaccine:	TAB - VACCINE.	19 - BA - Strain Vaccine: IN RUSSIA - only.
13/ Prevention:	a/ Detection and treatment of Carriers b/ Pasteurization of MILK.	a/ Pasteurization of milk b/ immunization of animals.
14/ Carriers:	— YES.	— NO human Carriers.

Brucellosis



Brucellosis. A calcified *Brucella* granuloma in the spleen of a man with fever of several years' duration. *Brucella* organisms that survive the action of polymorphonuclear leukocytes are ingested by macrophages and become localized in the organs of the reticuloendothelial system.

INFLUENZA-LIKE SYNDROME.

Brucellosis (undulant fever) presents with **FLU** and fever of variable duration, headache, sweats, arthralgia, and weakness.

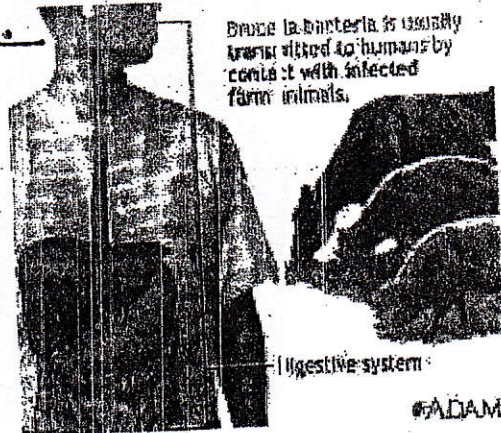
Osteoarticular (sacroiliitis) complications in 20-60%.

Genitourinary (orchitis and epididymitis) complications in 2-20%.

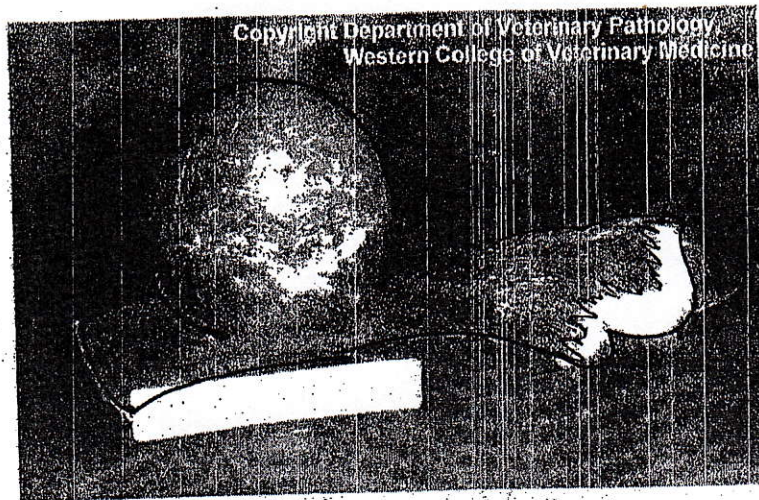
Incubation Period: 5-60 days

Natural risk to humans is from untreated milk and milk products, meat processing, and livestock handling.

Case Fatality Rate is about 2 % (endocarditis)

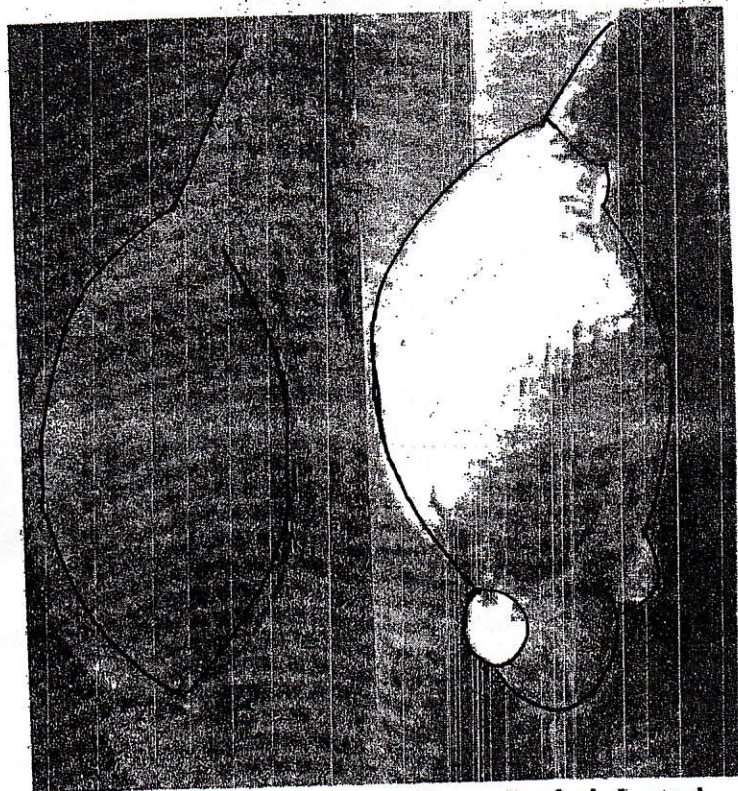


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Swollen "knee" joint of caribou due to brucellosis.

فيما الرتبة



Healthy ovine testicle

Brucellosis infected testicle

فيما الرتبة

Habitat and Transmission: Habitat is the human colon, where it is the predominant anaerobe. Transmission occurs by spread from the colon to the blood or peritoneum.

Pathogenesis: Lipopolysaccharide in cell wall is chemically different from and less potent than typical endotoxin. No exotoxins known. Capsule is antiphagocytic. Predisposing factors to infection include bowel surgery and penetrating abdominal wounds.

Laboratory Diagnosis: Gram-stained smear plus anaerobic culture. Identification based on biochemical reactions and gas chromatography. Serologic tests not useful.

Treatment: Metronidazole, clindamycin, and cefoxitin are all effective. Abscesses should be surgically drained. Resistance to penicillin G, some cephalosporins, and aminoglycosides is common. Plasmid-encoded β -lactamase mediates resistance to penicillin.

Prevention: In bowel surgery, perioperative cefoxitin can reduce the frequency of postoperative infections. No vaccine is available.

GRAM-NEGATIVE RODS ASSOCIATED PRIMARILY WITH THE RESPIRATORY TRACT

Haemophilus influenzae :-

Diseases: Meningitis, otitis media, and pneumonia are common.

Characteristics: Small gram-negative (coccobacillary) rods. Type b capsule is polyribitol phosphate. Requires factors X (hemin) and V (NAD) for growth.

Habitat and Transmission: Habitat is the upper respiratory tract. Transmission is via respiratory droplets.

Pathogenesis: Polysaccharide capsule is the most important determinant of virulence; 95% of invasive disease is caused by capsular type b. IgA protease is produced. Most cases of meningitis occur in children under 2 years of age, because maternal antibody has waned and the immune response of the child can be inadequate. No exotoxins identified.

Laboratory Diagnosis: Gram-stained smear plus culture on chocolate agar. Growth requires both factors X and V. Determine serotype by using antiserum in various tests, eg, immunoelectrophoresis, or latex agglutination. Capsular antigen present in serum or cerebrospinal fluid. Serologic test not useful.

Treatment: Ceftriaxone is the treatment of choice for meningitis.

Prevention: Rifampin can prevent meningitis in close contacts. Vaccine containing the type b capsular polysaccharide conjugated to diphtheria toxoid or other protein is given between 2 and 18 months of age.

Legionella pneumophila

Disease: Legionnaires' disease ("atypical" pneumonia).

Characteristics: Gram-negative rods, but stain poorly with standard Gram's stain. Require increased iron and cysteine for growth in culture.

Habitat and Transmission: Habitat is environmental water sources. Transmission is via aerosol. Person-to-person transmission does not occur.

Pathogenesis: Aside from endotoxin, no toxins, enzymes, or virulence factors are known. Predisposing factors include being over age 55 years, smoking, and having a high alcohol intake. Immunosuppressed patients, eg, renal transplant recipients, are highly susceptible.

Laboratory Diagnosis: Microscopy with silver impregnation stain or fluorescent antibody. Culture on charcoal yeast extract agar containing increased amounts of iron and cysteine. Diagnosis usually made serologically.

Treatment: Erythromycin.

Prevention: No vaccine or prophylactic drug is available.

Bordetella pertussis

Disease: Whooping cough (pertussis).

Characteristics: Small gram-negative rods.

Habitat and Transmission: Habitat is the human respiratory tract. Transmission is via respiratory droplets.

Pathogenesis: Two toxins are produced. Pertussis toxin stimulates adenylate cyclase by adding ADP-ribose onto the inhibitory coupling protein. Toxin has two components: subunit A, which has the ADP-ribosylating activity, and subunit B, which binds the toxin to cell surface receptors. In addition, extracellular adenylate cyclase is produced, which can inhibit killing by phagocytes.

Laboratory Diagnosis: Gram-stained smear plus culture on Bordet-Gengou agar. Identified by biochemical reactions and slide agglutination with known antisera. Serologic tests not useful.

Treatment: Erythromycin.

Prevention: The acellular vaccine containing purified proteins is recommended rather than the killed vaccine, which contains whole organisms. Usually given to children in combination with diphtheria and tetanus toxoids.

GRAM-NEGATIVE RODS CAUSING ZONOSEs

***Brucella* species (eg, *B. abortus*, *B. suis*, *B. melitensis*)**

Disease: Brucellosis (undulant fever).

Characteristics: Small gram-negative rods.

Habitat and Transmission: Habitat is domestic livestock. Transmission is via unpasteurized milk and cheese, or direct contact with the infected animal.

Pathogenesis: Organisms localize in reticuloendothelial cells. Virulence associated with intracellular survival. Endotoxin necessary for pathogenesis. No exotoxin or capsule identified. Predisposing factors include consuming unpasteurized dairy products and working in an abattoir.

Laboratory Diagnosis: Gram-stained smear plus culture on blood agar plate. Identified by biochemical reactions and by agglutination with known antiserum. Diagnosis may be made serologically also.

Treatment: Tetracycline.

Prevention: Pasteurize milk; vaccinate cattle. No human vaccine is available.

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